Hippocampal Contribution to Renewal of Conditional Fear After Extinction

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INTRODUCTION

•The hippocampus is vital to the formation of an integrated, Gestalt-type representation of an environment/context (Fanselow, 2000).

•Previous studies from our lab have implicated the dorsal hippocampus (DH) in the formation and short-term storage of contextual fear memories (Kim and Fanselow, 1992).

•Deficits in context-sensitive tasks/effects (e.g. Morris Water Maze, context pre-exposure effect, trace conditioning, context conditioning) can occur when the hippocampus is compromised (e.g., see Cushman, Moore, Olson & Fanselow, Poster #874.8/RR43).

•In *renewal*, conditional responding (CR) to a previously extinguished conditional stimulus (CS) returns when the CS is tested outside of the extinction context (see Bouton, 2004). Given the context-sensitivity of renewal, the DH is a natural candidate for investigation of the brain structure(s) underlying renewal.

•Previous studies interested in the role of the DH in renewal have yielded mixed results. Some demonstrate that lesions or inactivations of DH (regardless of timing or renewal type) result in deficits in fear renewal (Corcoran & Maren 2001, 2004; Ji & Maren, 2005), while another study (Frohardt et al., 2000) finds no renewal deficit in animals with DH lesions (pre-training).

•One notable difference between these findings is in how fear renewal is analyzed (i.e. initial vs. average CR to a CS). This difference suggests that the role of the DH in renewal may depend on *when* during the time course of renewal, conditional fear is assessed.

•Indeed, previous findings from our lab (Anagnostaras et al., 1999) showed that hippocampal lesions are sensitive to the time course of a test session, as lesions of the hippocampus eliminate *overall* fear responding to a context conditioned in the recent past, but leave *initial* contextual fear intact.

QUESTION 1: Does the DH play a necessary role in renewal, and if so, is this role dependent on the time course of renewal?

•Given a time-selective role for the DH in renewal, we asked whether another structure, in complement to the DH, is responsible for the initial moments of fear renewal that DH lesions leave intact.

•Recent investigations implicating the medial prefrontal cortex (mPFC) (infralimbic (IL) region in particular) in fear extinction (see Quirk & Mueller, 2008), led us to investigate whether or not the mPFC could be the structure complementing the DH in renewal.

QUESTION 2: What role does the mPFC play in fear renewal?

HISTOLOGY

Upon termination of behavioral testing, rats were overdosed and perfused intracardially with KPBS and 4% paraformaldehyde. Brains were cryoprotected (30% sucrose solution), frozen (-20 C), sectioned (50 μm), mounted, and stained for cell bodies (cresyl violet). Using a light microscope, lesions were verified and images were captured.

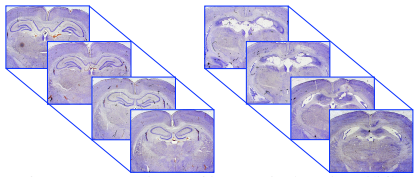


Figure 1. Representative coronal sections of the DH in a sham lesioned animal (left) compared to a DH lesioned animal (right). Animals with lesions sparing the CA1 region of the DH were excluded.

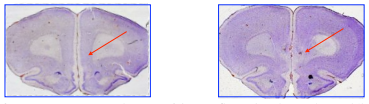
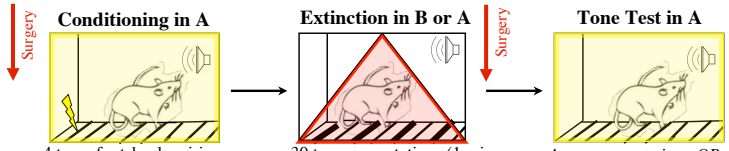


Figure 2. Representative coronal section of the mPFC in a sham lesioned animal (left) compared to a mPFC lesioned animal (right). All animals included in our analyses suffered damage to the IL region of mPFC. In some animals, this damage extended to the prelimbic (PL) region. Animals with only PL damage were excluded.

METHODS



Subjects: Naïve adult male Long-Evans rats, initially weighing 250-300 grams, handled for 5 days prior to the start of the experiment.
Design: (See diagram left). Stimuli: Tone-CS (30 sec, 80 dB, 2800 Hz); Footshock-US (2 sec, .9 mA).
Surgery: DH lesions (excitotoxic): NMDA (.4 μl/site) was infused at a rate of 0.1 μl per minute. A-P -2.8, 4.2; M-L +/- 1.6, 2.6; D-V 3.5. mPFC lesions (excitotoxic): NMDA (.2 μl/site) was infused at a rate of 0.1 μl per minute. Lesions were aimed at the IL region of mPFC. A-P +2.9; M-L +/- .5; D-V 4.9.
Measurement of fear: Conditional fear was indexed by % freezing. Behavior was recorded using near infrared (NIR) video tracking equipment and computer software (VideoFreeze, Med-Associates Inc.) Activity was scored by assessing noise of pixelation of video images taken at 30 frames per second. Freezing was defined as sub-threshold activity for longer than 1 s.

RESULTS

EXPERIMENT 1a: Pre-training DH lesions attenuate *average* fear renewal

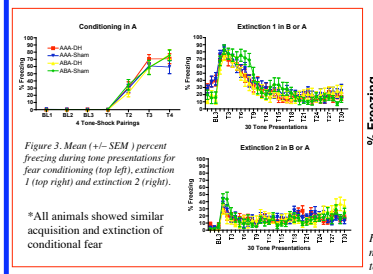


Figure 3. Mean (+/– SEM) percent freezing during tone presentations for fear conditioning (top left), extinction 1 (top right) and extinction 2 (right).

*All animals showed similar acquisition and extinction of conditional fear

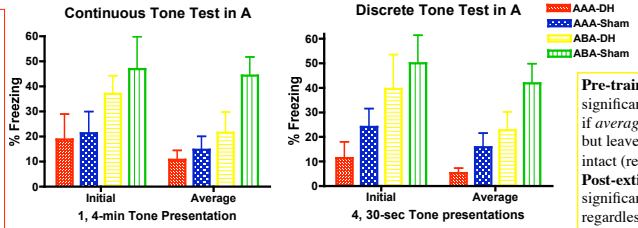


Figure 4. Mean (+/– SEM) percent freezing during 1, 4 min tone presentation. Freezing to the initial 30 s of the tone is compared to freezing averaged across all 4 minutes.

Figure 5. Mean (+/– SEM) percent freezing during 4, 30 s tone presentations. Freezing to the initial tone is compared to freezing averaged across all 4 tones.

Pre-training DH lesions significantly attenuate renewal if *average* freezing is analyzed, but leave initial fear expression intact (regardless of test type).
Post-extinction DH lesions significantly attenuate renewal, regardless of whether initial or average levels of freezing are analyzed.

EXPERIMENT 1b: Post-extinction DH lesions attenuate fear renewal

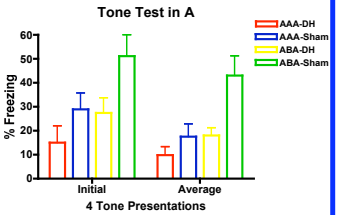


Figure 6. Mean (+/– SEM) percent freezing during 4 tone presentations. Freezing to the initial tone is compared to freezing averaged across all 4 tones.

EXPERIMENT 2: Pre-training mPFC lesions enhance spontaneous recovery

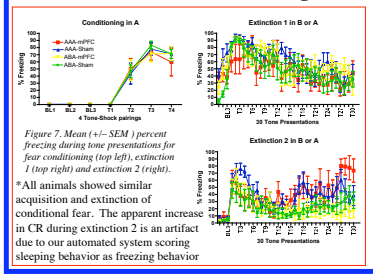


Figure 7. Mean (+/– SEM) percent freezing during tone presentations for fear conditioning (top left), extinction 1 (top right) and extinction 2 (right).

*All animals showed similar acquisition and extinction of conditional fear. The apparent increase in CR during extinction 2 is an artifact due to our automated system scoring sleeping behavior as freezing behavior

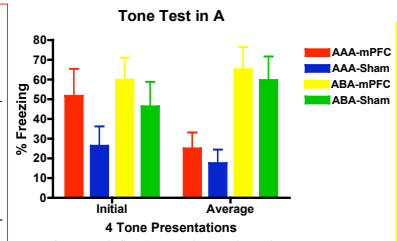


Figure 8. Mean (+/– SEM) percent freezing during 4 tone presentations. Freezing to the initial tone is compared to freezing averaged across all 4 tones.

Pre-training mPFC lesions do not attenuate renewal, regardless of whether initial or average freezing levels are analyzed. Interestingly, amongst AAA trained animals, those with mPFC lesions exhibit a significant increase in spontaneous recovery compared to sham controls, which is present during initial fear expression but not during average fear expression.

Context Fear in animals with mPFC lesions

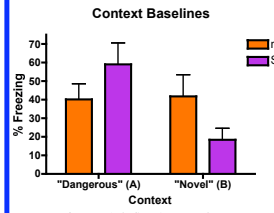


Figure 9. Mean (+/– SEM) percent freezing to context taken from average freezing during 3 min baseline period preceding extinction session 1.

Animals with damage to mPFC show a deficit in context discrimination, as opposed to sham animals (left). Consistent with prior results from our lab (Wiltgen et al. 2006), there was no effect on context fear produced by pre-training DH lesions and multiple shocks (data not shown).

CONCLUSIONS

• Pre-training DH lesions only attenuate renewal when analyses are performed across *average* percent freezing during a test session. This suggests that the DH is important for renewed responding as a test session progresses, but that initial responding is supported by another structure.
• Post-extinction DH lesions eliminate both average *and* initial renewal. Thus, it is likely that the structure partially compensating for the DH after pre-training lesions is recruited during conditioning and/or extinction. Further experiments comparing post-conditioning and post-extinction DH lesions would reveal exactly when this alternate structure is required.
• Experiment 1 demonstrates that fear renewal cannot be fully explained by the functioning of one structure or by a simple shift in context. Instead, these data suggest that renewal is comprised of two mechanisms: one that is DH-dependent and enables overall renewed responding, and another, which is DH-independent and enables initial responding. Whether or not this latter mechanism is even context sensitive - and uses contextual information in a manner similar to the DH - remains open to further investigation.
• Experiment 2 demonstrates that the mPFC is *not* required for fear renewal.
• However, Experiment 2 shows that the mPFC *is* required for decreasing initial fear responding in a "safe" (i.e. extinction) context. This finding fits with recent research suggesting that the mPFC (specifically the IL region) plays an important, inhibitory role in extinction (see Quirk & Mueller, 2008).
• Animals with mPFC lesions also show enhanced fear to a novel context and reduced fear to a dangerous context compared to sham animals. This suggests that the mPFC may be involved in modulating responses when an animal has to discriminate between dangerous and safe environments.
• Together, these experiments demonstrate that while both the DH and mPFC are important in processing contextual information, they perform unique roles that depend in large part on the time course of a test session.

REFERENCES

Anagnostaras S.G., Maren S., Fanselow M.S., 1999. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *Journal Neuroscience, 19(3)*:1106-14.
Bouton, M. E., 2004. Context and behavioral processes in extinction. *Learning and Memory, 11*, 485-494. Kim J. J., Fanselow M. S., 1992. Modality-specific retrograde amnesia of fear. *Science, 256*, 675-677.
Corcoran K. A., Maren S., 2001. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *Journal Neuroscience 21*, 1720–1726.
Corcoran K. A., Maren S., 2004. Factors regulating the effects of hippocampal inactivation on renewal of conditional fear after extinction. *Learning and Memory, 11(5)*:598-603.
Fanselow MS. 2000. Contextual fear, gestalt memories, and the hippocampus. *Behav. Brain Research 110*, 73–81.
Frohardt RJ, Guarraci FA, Bouton ME., 2000. The effects of neurotoxic hippocampal lesions on two effects of context after fear extinction. *Behavioral Neuroscience 114*, 227–240.
Ji J, Maren S., 2005. Electrolytic lesions of the dorsal hippocampus disrupt renewal of conditional fear after extinction. *Learning and Memory 12*, 270–276.
Quirk G.J., Mueller D., 2008. Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology 33(1)*: 56-72.
Wiltgen B.J., Sanders M.J., Anagnostaras S.G., Sage J.R., Fanselow M.S., 2006. Context fear learning in the absence of the hippocampus. *J Neuroscience, 17*, 26(20):5484-91.

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